May 17, 1993 SMALL DNA VIRUSES Harold Varmus

<u>General Reference and Review Articles</u>

Relevant chapters in Fields et al. *VIROLOGY*, 1990.

Challberg and Kelly, Animal virus DNA replication. Ann. Rev. Biochem. 58:671, 1989.

Fanning. Simian virus 40 large T antigen: The puzzle, the pieces, and the emerging picture. J. Virol. 66:1289, 1992.

Hurwitz et al. The in vitro replication of DNA containing the SV40 origin. J.Biol.Chem. 265:18043, 1990.

Matthews and Shenk. Adenovirus virus-associated RNA and translational control. J.Virol. 65:5657, 1991.

Prives. The replication functions of SV40 T antigen are regulated by phosphorylation. Cell 61:735, 1990.

<u>A Selection of Important Original Papers</u>

(Also see papers assigned for Discussion Groups on Papilloma virus replication.)

Challberg and Kelly. Adenovirus DNA replication in vitro. PNAS 76:655, 1979.

Dana and Nathans. Bidirectional replication of simian virus 40 DNA. PNAS 69:3097, 1972.

Lambert et al. A transcriptional repressor encoded by BPV-1 shares a common carboxyl-terminal domain with the E2 transactivator. Cell 50: 69, 1987.

Li and Kelly. Simian virus 40 DNA replication in vitro. PNAS 81:6973, 1984.

Samulski et al. Targeted integration of AAV into human chromosome 19. EMBO J. 10:3941, 1991.

Shortle et al. Mutational analysis of the SV40 replicon: Pseudorevertants of mutants with a defective replication origin. PNAS 76:6128, 1986.

Stewart et al. Image reconstruction reveals the complex molecular organization of adenovirus. Cell 67:145, 1991.

Tamanoi and Stillman. Initiation of adenovirus DNA replication in vitro requires a specific DNA sequence. PNAS 80:6446, 1983.

Tsurimoto et al. Sequential initiation of lagging and leading strand synthesis by two different polymerase complexes at the SV40 DNA replication origin. Nature 346:534, 1990.

Weinberg et al. Reconstitution of simian virus 40 DNA replication with purified proteins. PNAS 87:8692, 1990.

COMPARATIVE VIROLOGY: A PREVIEW

	Parvos	Polyomas	Papillomas	Adenos
Particle	NNC	NNC	NNC	NNC (COMPLEX)
Genome	SS DNA LINEAR 5KB	DS DNA CIRC. 5KB	DS DNA CIRC. 8KB	DS DNA LINEAR + PROT. ~40KB
Replication strategy	HOST POL. HAIRPIN PRIMERS	HOST POL. T Ag ORI	HOST POL. E1, E2 ORI	VIRAL POL PROT. PRIMER TERM. ORI'S
Gene expression	ALT. PROM. + SPLICING	EARLY & LATE BIDIRECTIONAL TRANSCR.	COMPLEX EARLY LATE	ALL MECHS. IE → E → L
Peculiarities of virus growth	HOST OR HELPER DEPENDENT	PERM. vs. NP CELLS	LATE, VEGETATIVE IN DIFF. KERATINOCYTES	PERM. vs. NP CELLS
Utility	VECTOR? (ONCOLYTIC)	VECTOR (EXPT'AL)	VECTOR (EXPT'AL USE)	VECTOR FOR CF?

PARVOVIRUSES

PATHOGENIC AGENTS: B19 VIRUS (ANEMIA OF NEONATES)

MANY VETERINARY DISEASES

GROWTH DEPENDENT UPON HELPER VIRUS (ADENOVIRUS OR HERPESVIRUS FOR ADENOASSOCIATED VIRUSES) OR S PHASE (AUTONOMOUS PARVOS, SUCH AS MVM, H1,ADV)

PARTICLE: ICOSAHEDRAL (CRYSTAL STRUCTURE)

THREE STRUCTURAL PROTEINS (+ "REP" PROTEIN AND DNA)

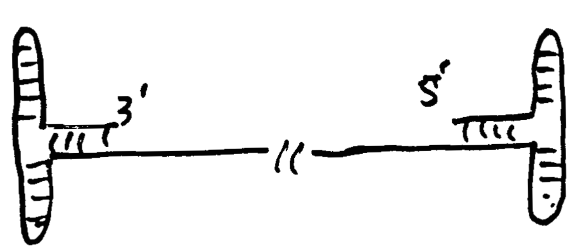
GENOME IS SINGLE STRANDED DNA OF CA. 5 KB

MINUS **OR** PLUS STRANDS (AAV)

MINUS ONLY (MVM)

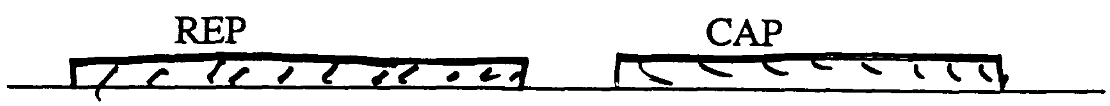
PALINDROMIC ENDS (100-300 NT)

E.G.



<u>CODING REGIONS AND GENE EXPRESSION</u>

TWO LARGE NON-OVERLAPPING FRAMES

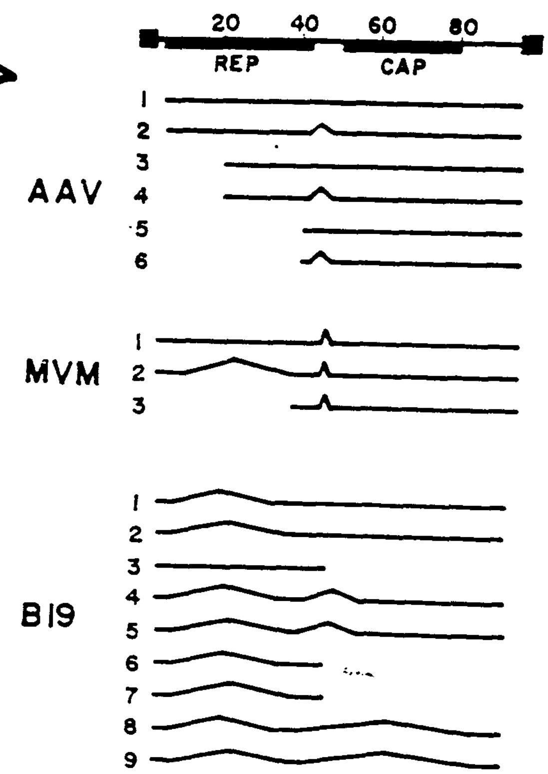


EXPRESSED VIA 1 TO 3 PROMOTERS AND ALTERN. SPLICING

REP PROTEIN AS TRANSACTIVATOR??

LINKED TO DNA (IN VIRION)

ROLE IN REPLICATION??



Transcriptional maps of three parvoviruses

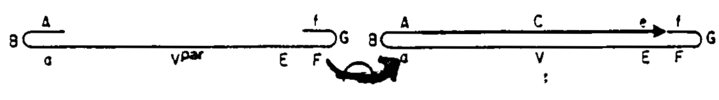
PARVOVIRUSES:

REPLICATION STRATEGY

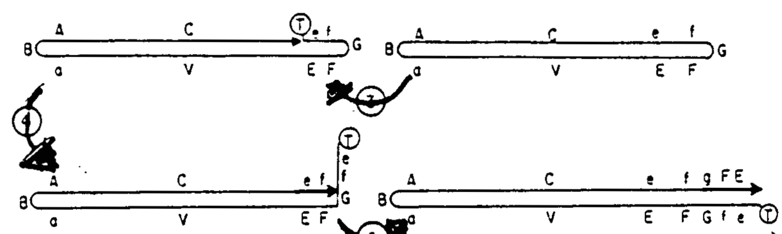
- REQUIRES S PHASE, BUT DOES NOT INDUCE IT
- USES HOST DNA POLYMERASES

THE PRIMER/TELOMER PROBLEM:

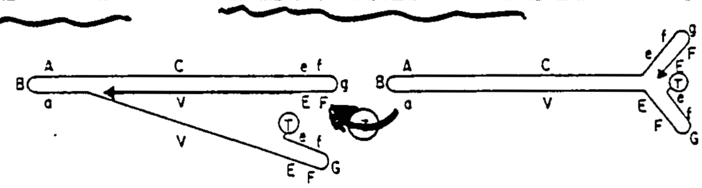
PALINDROMIC ENDS PROVIDE DNA PRIMER



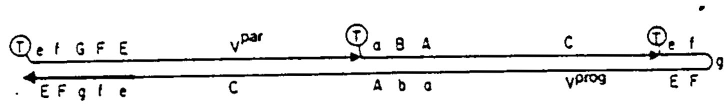
CLEAVAGE OF HAIRPINS, EXTENSION REGENERATES ENDS



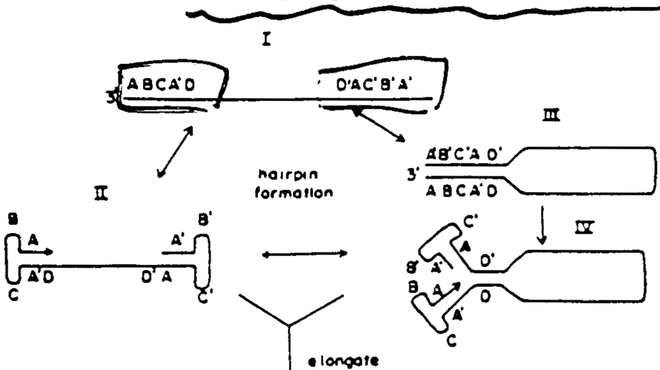
SECOND STRAND BY DISPLACEMENT SYNTHESIS

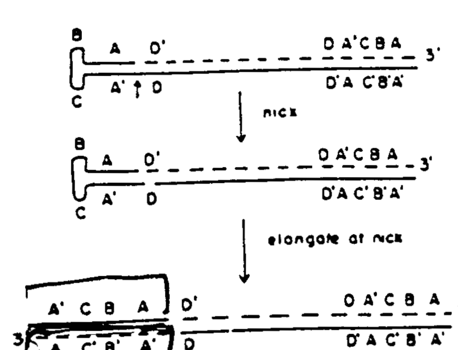


ROLLING HAIRPIN IN SOME CASES



FLIP-FLOP OF PALINDROMES





Model for AAV DNA replication.

AAV: LATENT INFECTION, INTEGRATION, VECTORS

LATENT STATE FREQUENT AFTER HELPER-FREE INFECTION

GENOME INTEGRATED IN TANDEM,

OFTEN ON CHR. 19 Q 13.4-TER WITHIN 100 BP

REP GENE REQUIRED FOR ADENO RESCUE, NOT FOR LATENCY

(NOT AN INTEGRASE)

RESCUE MIMICKED BY RECOVERY OF GENOME FROM

RECOMBINANT PLASMIDS IN TRANSFECTED CELLS

AND IN CELL EXTRACTS

AAV VECTORS: ONLY ENDS REQUIRED

HIGH EFFICIENCY

ADVANTAGE OF CHR. 19 TARGETING?

LIMITED CARRYING CAPACITY

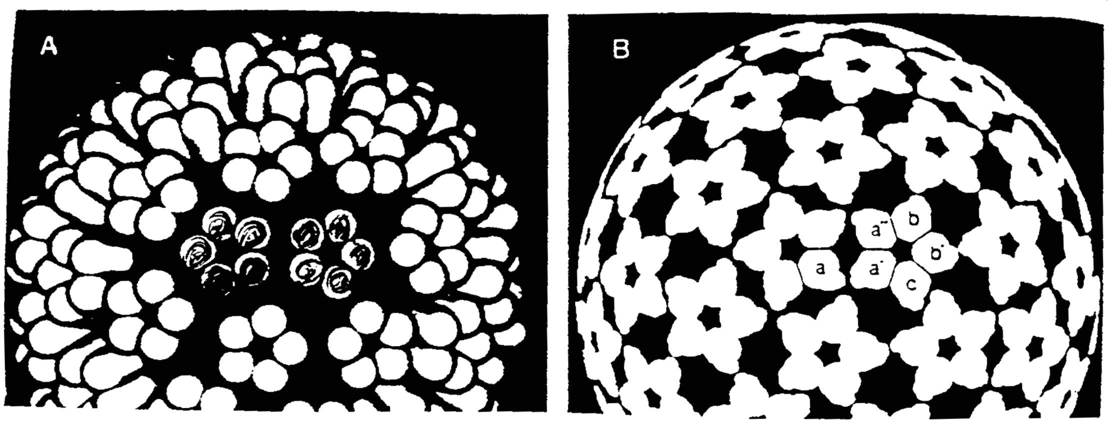
HELPER CELLS AVAILABLE

POLYOMA VIRUSES

EXAMPLES AND PATHOLOGY

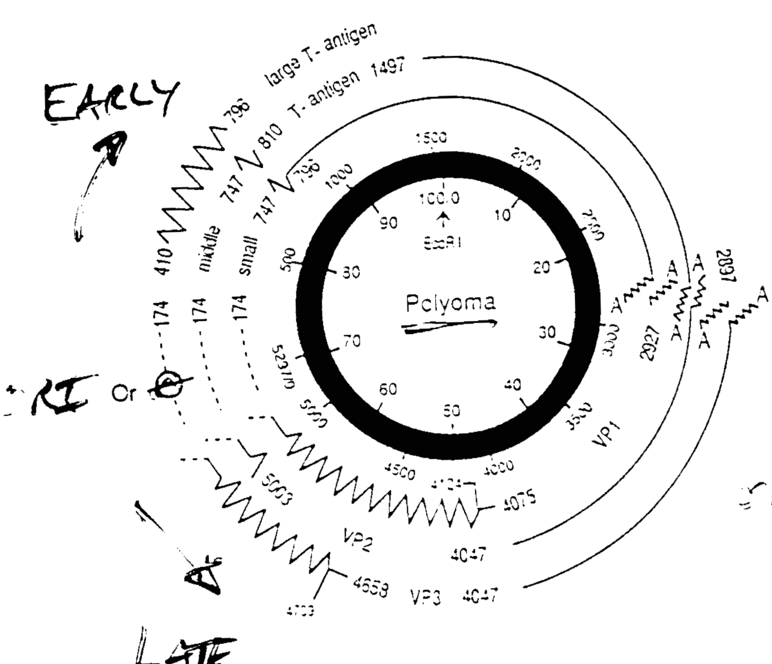
Virus	Host species	
Polyomavirus	Mouse	TUMORS (MICE, ETC)
K papovavirus	Mouse	
Hamster papovavirus (HapV)	Hamster	
Simian vacuolating virus 40 (SV40)	Monkey	TUMORS (RODENTS)
Lymphotropic papovavirus (LPV)	Monkey	
Simian agent 12 (SA12)	Baboon	
BKV	Human	
JCV	Human	PML (HUMANS)
Rabbit kidney vacuolating virus (RKV)	Rabbit	
Budgerigar fledgling disease virus	Budgerigar	(A BIRD!)

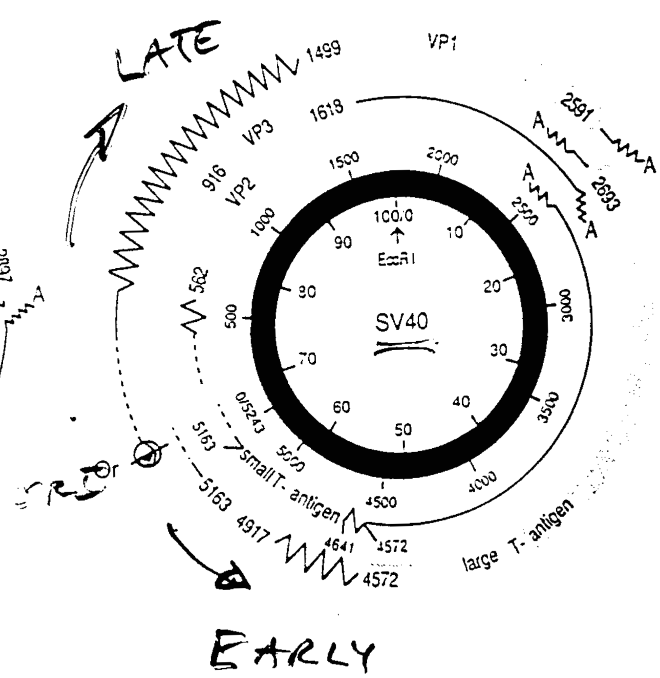
STRUCTURE OF PARTICLE (PSEUDO-EQUIVALENCE)



CRYSTAL STRUCTURE NOW AVAILABLE

GENOME LAYOUT





OVERVIEW OF LIFE CYCLE

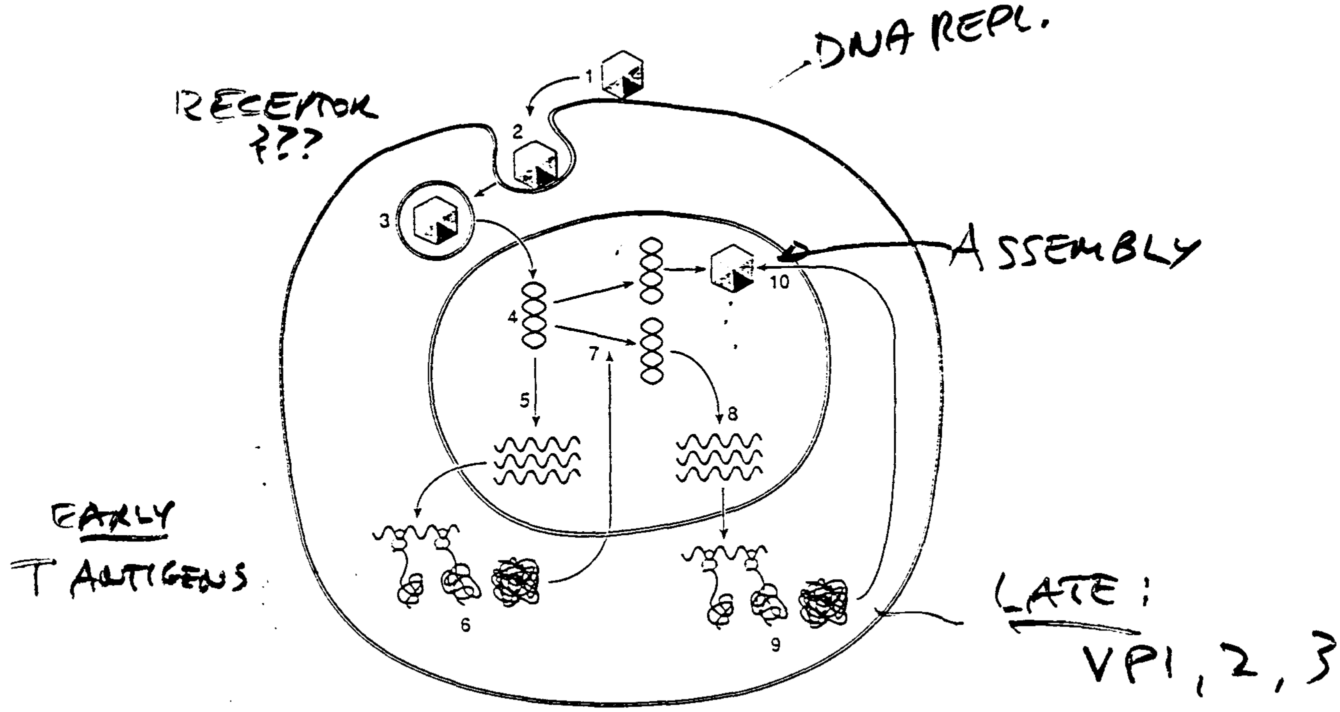


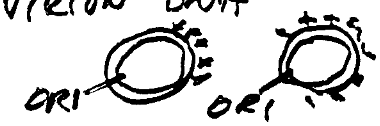
FIG. 3. Replication cycle of polyoma and SV40. Steps in the replication cycle are indicated by numbers as follows: 1, adsorption of virions to the cell surface; 2, entry by endocytosis; 3, transport to the cell nucleus; 4, uncoating; 5, transcription of early region mRNAs; 6, translation of early proteins (T antigens); 7, viral DNA replication; 8, transcription of late region mRNAs; 9, translation of late proteins (virion proteins); 10, assembly of progeny virus particles.

GREAT MOMENTS IN HISTORY OF SV40

- DISCOVERY (IN POLIO VACCINE)
- DNA IS CIRCULAR AND SUPERCOILED
- DEFINITION OF ORIGIN

  VIRION DNA

  PULSE-CHASE + RESTRICTION MAPPING

  ORI BUBBLE IN EM + RESTRICTION MAPPING

- GENETIC STRATEGIES:
  - TS-A : DEFINE ROLE OF TAg IN { START OF REPL. / REPRESSION OF EARLY / TRANSFORM. }
  - MARKER RESCUE : DEFINE TAg CODING REGION
  - PSEUDOREVERTANTS : TAg MUTANTS SUPPRESS ORI MUTANTS
- EARLY AND LATE RNAS FROM DIFFERENT STRANDS ·
- ENHANCERS
- INTEGRATION OF VIRAL DNA (ALBEIT IRREGULAR)
- HELPER CELLS (COS CELLS: CV-1 WITH ORI MUTANT DNA)
- IN VITRO DNA SYNTHESIS: T AG AND ORI DEPENDENCE
- HOST DEPENDENCE (MONKEY VS. RODENT CELLS)

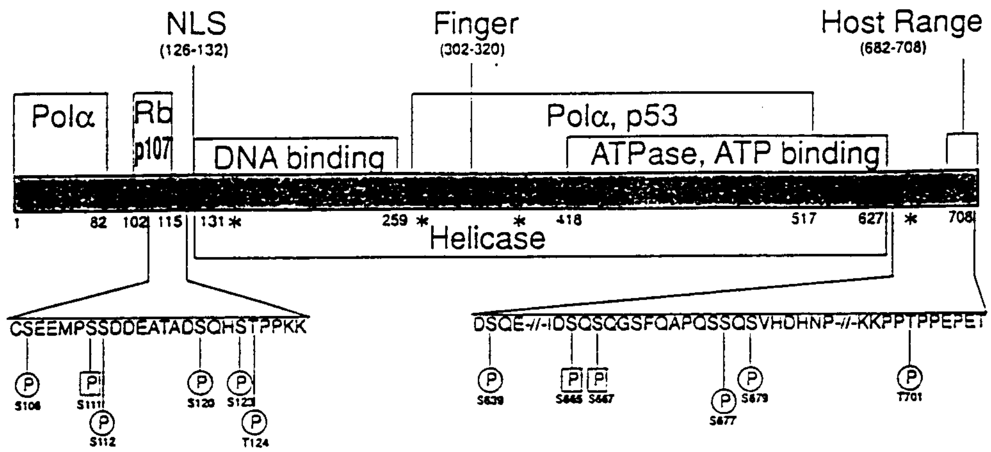
  MONKEY ↓ PERMISSIVE ↓ VIRUS PROD

  RODENT ↓ NON PERMISSIVE ↓ TRANSFORMATION

ALSO GENETIC VECTORS IN ANIMAL CELLS

ETC

MULTIPLE ROLES OF T ANTIGEN



PHYSIOLOGY: STIMULATE HOST DNA SYNTHESIS

(TRANSFORM CELLS)

INHIBIT SYNTHESIS OF EARLY RNA

INITIATE SYNTHESIS OF VIRAL DNA

ENHANCE SYNTHESIS OF LATE RNA

HELPER FUNCTION FOR DEFECTIVE ADENO

BIOCHEMISTRY: BINDS TO ORIGIN (AS HEXAMER)

TARGET FOR PHOSPHORYLATION (MULTIPLE

KINASES) AND DEPHOSPH. (PP2A.....) (SEE DIAGRAM)

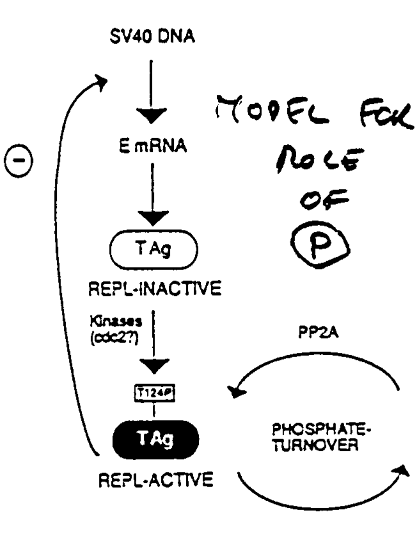
BINDS TO RB PROTEIN AND P53

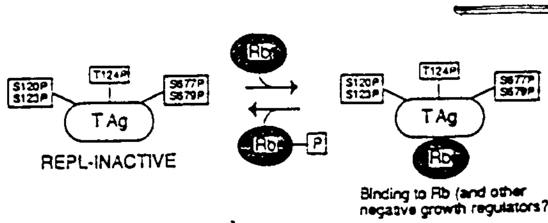
BINDS HOST-SPECIFIC DNA POL-ALPHA

NUCLEAR LOCALIZATION SIGNAL

ATP-DEPENDENT HELICASE

MODEL FOR ROLE OF P





REPLICATION OF SV40 DNA IN VITRO

LI AND KELLY: REPLICATION IN CRUDE EXTRACT

1984 ORI-DEPENDENT

T AG-DEPENDENT

BI-DIRECTIONAL AND FULL LENGTH

KELLY, STILLMAN, HURWITZ ET AL:

<u>REQUIRED FOR INITIATION</u>: ORI, T AG (BINDING, HELICASE)

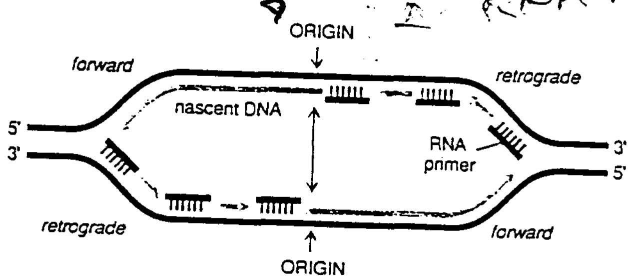
SS-DNA BP (RF-A, RP-A)

TAg BINDS UNWINDS

DNA POL ALPHA/PRIMASE

RNA PRIMERS

(STIM. BY PP2A, ACTS ON T AG)



<u>REQUIRED FOR ELONGATION:</u> DNA POL DELTA/PCNA

DNA-DEP. ATPASE (RF-C)

TOPOISOMERASE I

PAPILLOMA VIRUSES

MANY STRAINS IN MAN (OVER 60) AND ANIMALS

(FAVORED: BPV-1, HPV-16 AND -18, HPV-6 AND -11)

PATHOLOGY: BENIGN TUMORS (PAPILLOMAS) AND

MALIGNANT TUMORS (CARCINOMAS)

OF SKIN AND MEMBRANES

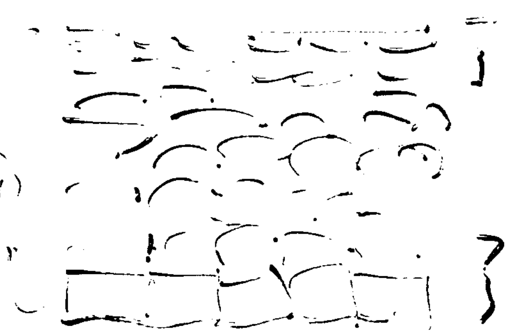
HUMAN CERVICAL CARCINOMA

GROWTH PROPERTIES: VIRUS PRODUCED ONLY IN DIFF.

KERATINOCYTES WHERE L1 AND L2 EXPRESSED

AND REPLICATION OF VIRAL DNA IS "VEGETATIVE"

SKIN
EPIDERM)



MATURE KERATINOCYTES — LATE GENES EXPR. VEGETATIVE REPL.

BASAL, STEM CELLS — EPISOMAL, LOWCOPY DN. NO LATE EXPR.

GENOME REPLICATES AS EPISOME IN SOME CELL LINES,

IN BASAL EPITHELIUM (MAINTAINED AS 50-400 COPIES).

TRANSCRIPTIONAL CONTROL BY PAPILLOMAVIRUSES

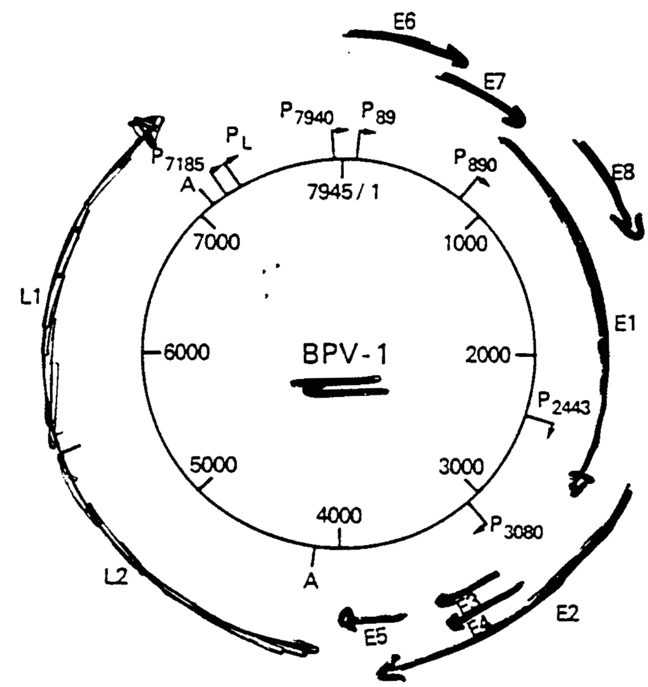
GENOME ORGANIZATION: EARLY AND LATE

8KB

DS CLOSED CIRCULAR DNA

UNIDIRECTIONAL TRANSCRIPTION

LATE



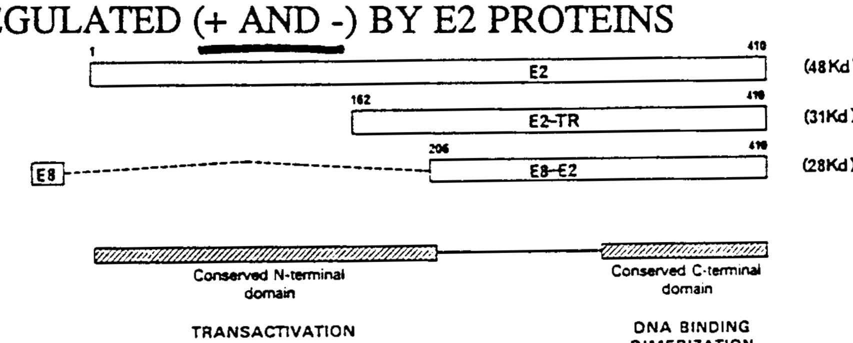
EARLY

The bovine papillomavirus type 1 genome.

MULTIPLE PROMOTERS

LONG CONTROL REGION:

REGULATED (+ AND -) BY E2 PROTEINS



ENHANCERS

REPRESSORS

MULTIPLE COPIES OF E2 BINDING SITE (ACCN6GGT)

EARLY REGION SUFFICIENT FOR TRANSFORMATION

(MECHANISMS TO BE DISCUSSED IN FINAL WEEK)

DNA REPLICATION

REQUIRES ORI AND E1 AND E2 PRODUCTS

HOST DNA POLYMERASE

(SEE DISCUSSION GROUPS)

INFLUENCE OF OTHER GENES ON COPY NUMBER?

ON VEGETATIVE REPLICATION IN KERATINOCYTES?

REPLICATION COORDINATED WITH S PHASE

INTEGRATION RARE EVENT

ADENOVIRUSES

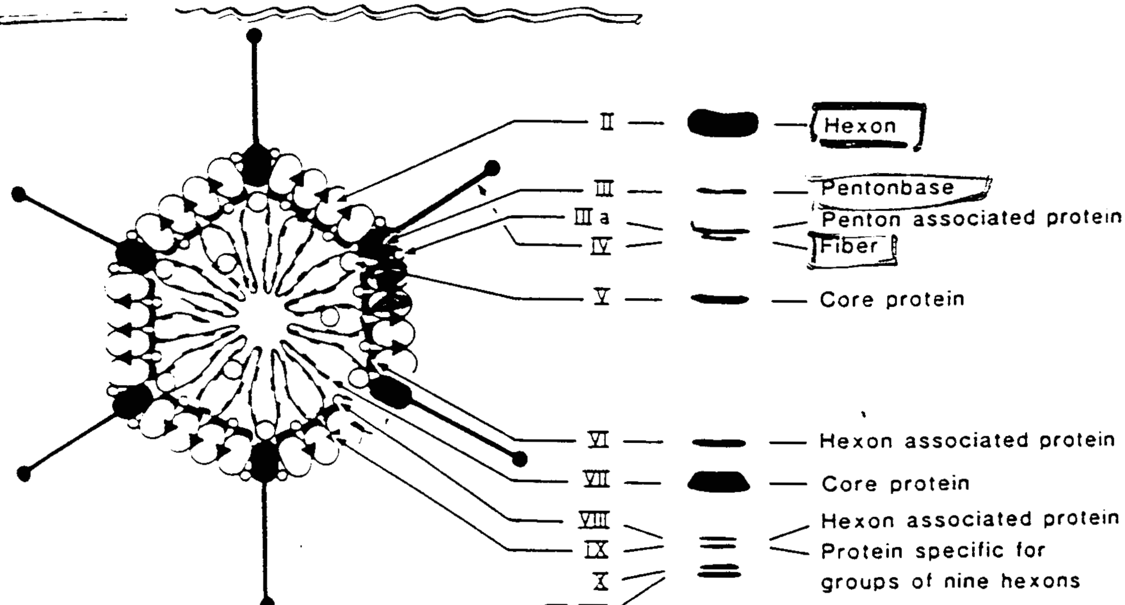
MANY STRAINS IN HUMANS AND OTHER MAMMALS, BIRDS

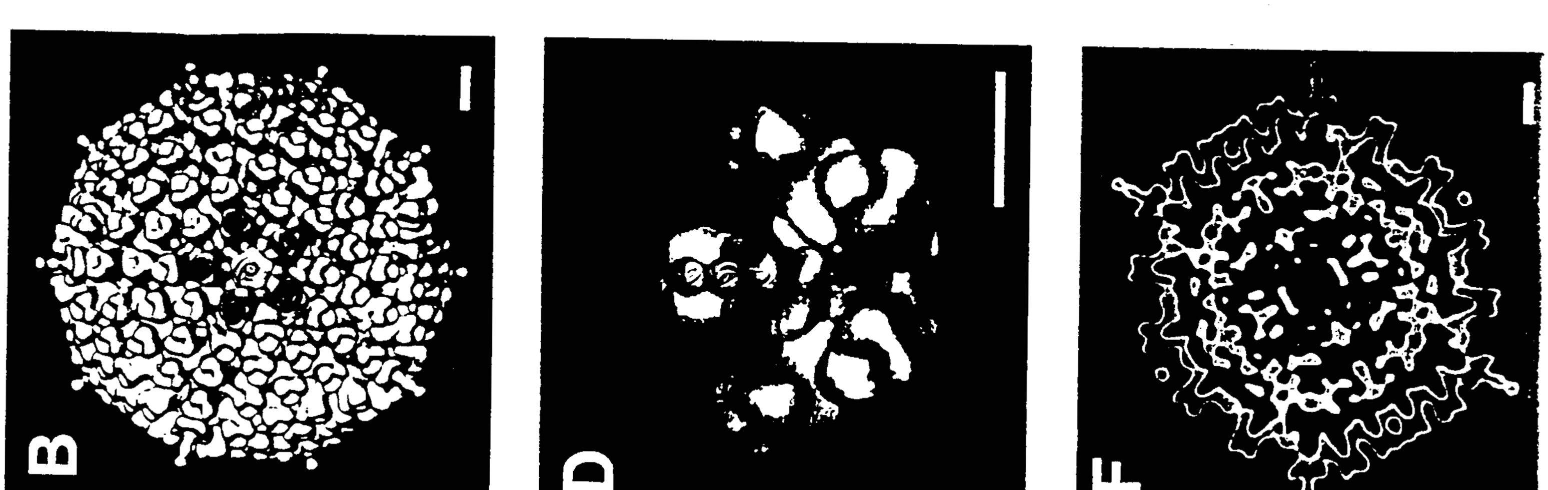
(BEST STUDIED: HUMAN ADENO 2, 5, 12, 18)

PATHOLOGY: PHARYNGITIS, CONJUNCTIVITIS

TUMORS (RODENTS INFECTED WITH HUMAN AD)

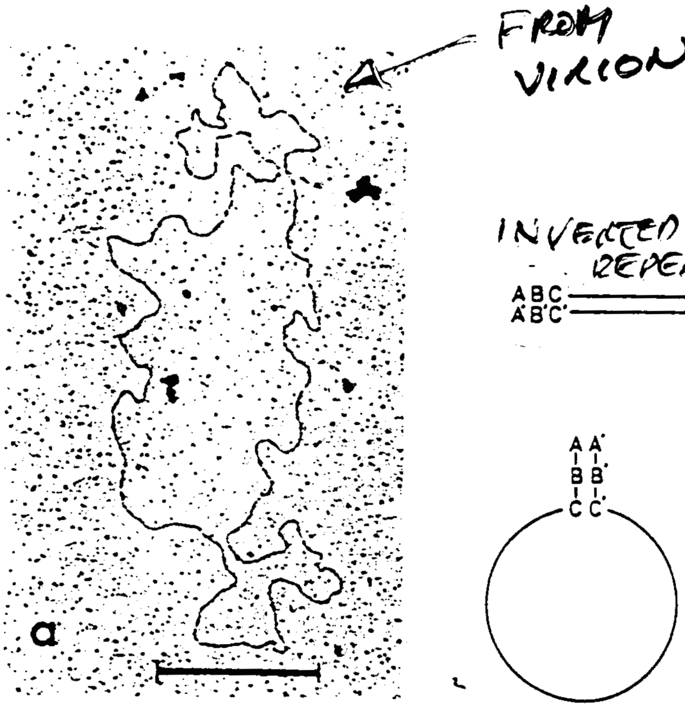
PARTICLE: COMPLEX NUCLEOCAPSID

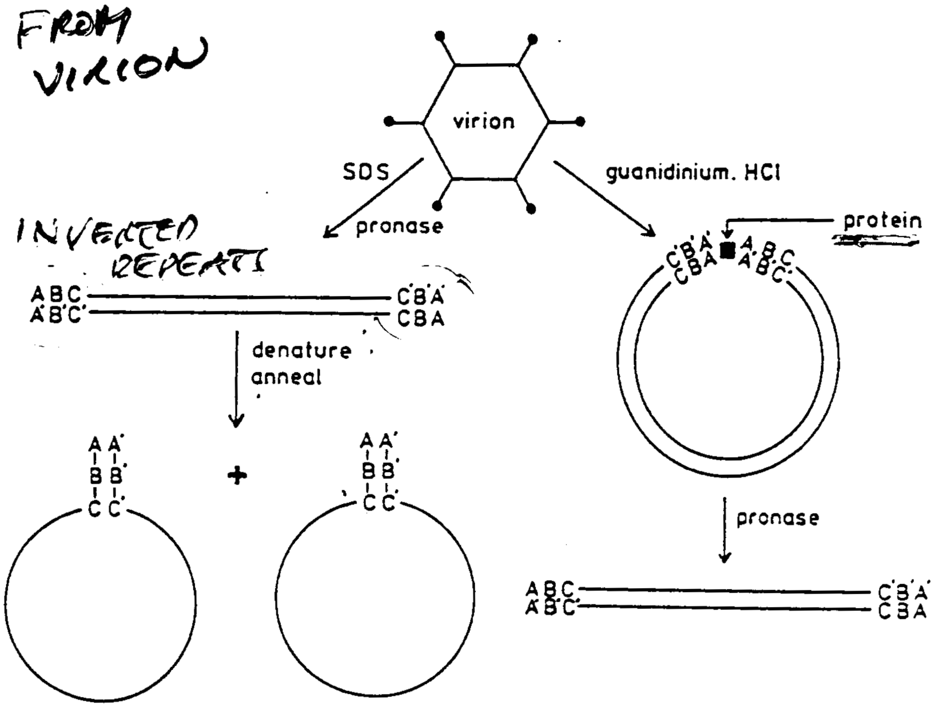


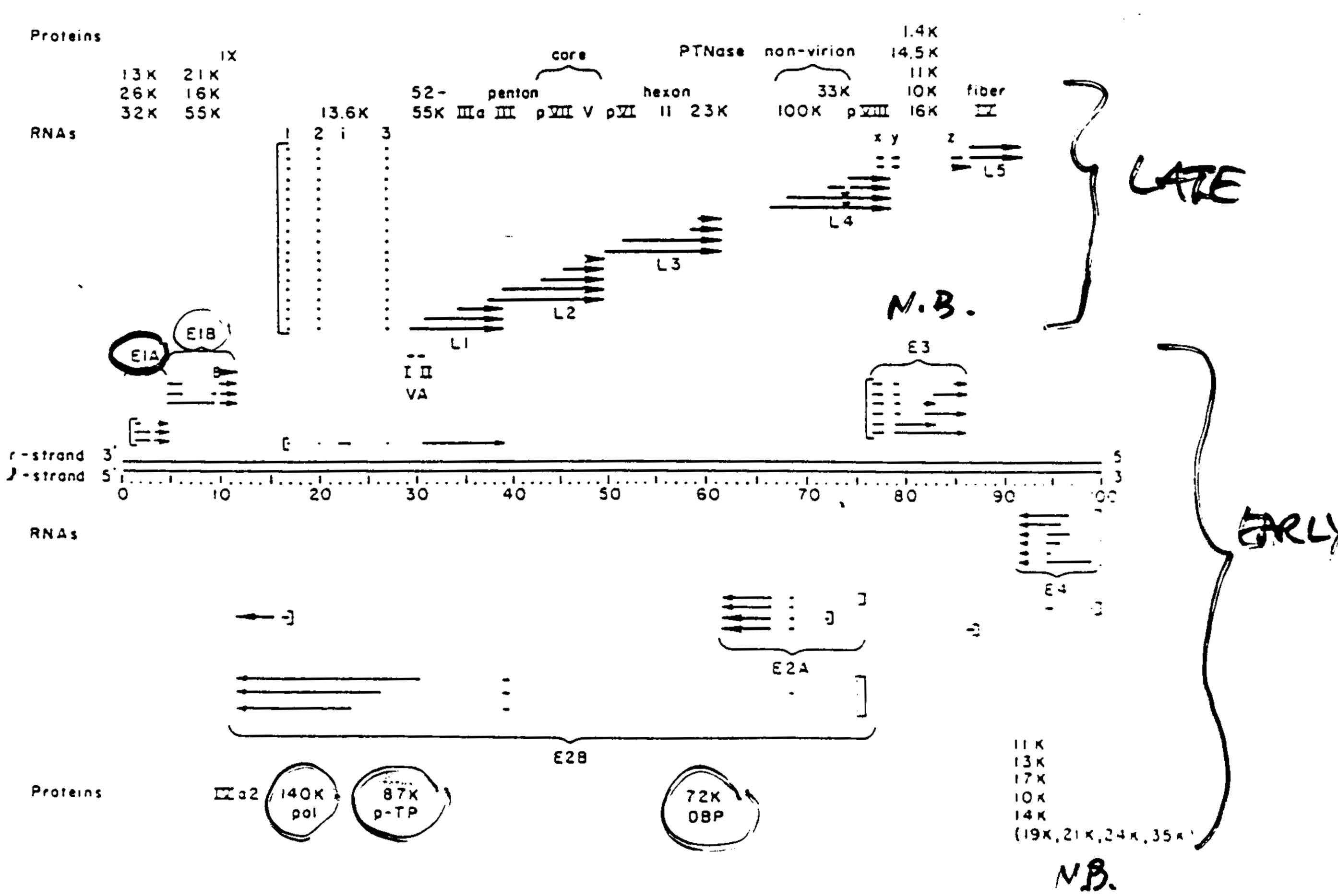


ADENOVIRUS GENOME

DS LINEAR DNA, CA. 40 KB, WITH TERM. PROTEIN







OVERVIEW OF ADENOVIRUS REPLICATION CYCLE

ENTRY VIA ENDOSOMES, RECEPTOR NOT KNOWN

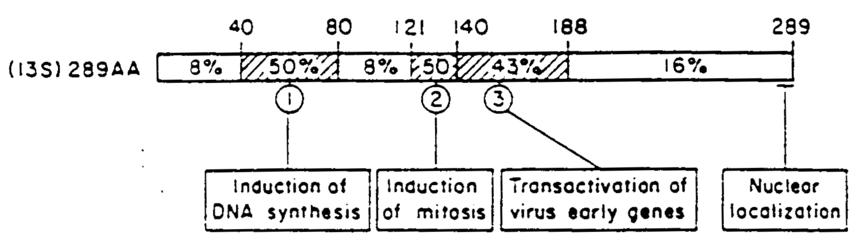
TEMPORALITY: IMMEDIATE EARLY (E1A)

EARLY (E1B, E2, E3, E4, MLP)

DNA REPLICATION

LATE (STRUCTURAL GENES VIA MLP)

E1A REGULATES OTHER EARLY GENES VIA E2F



(E1A REMOVES RESTRAINT IMPOSED BY pRB)

E2 ESSENTIAL FOR DNA REPLICATION: TERM PROT, DNA POL. (+72 kD SSDNA BP)

(E1A AND E1B ARE ONCOGENIC...SEE LATER LECTS.)

LATE M-RNAS (MONOCISTRONIC) MADE BY DIFF. SPLICING

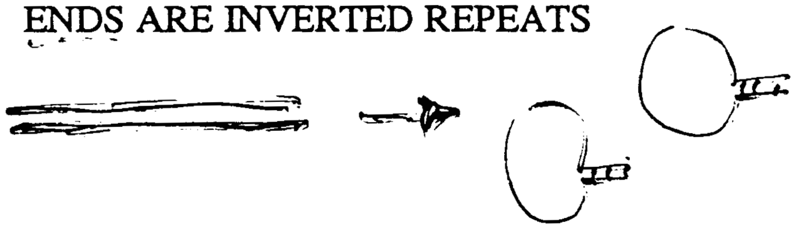
AND POLYADENYLATION FROM ONE PROMOTER-->

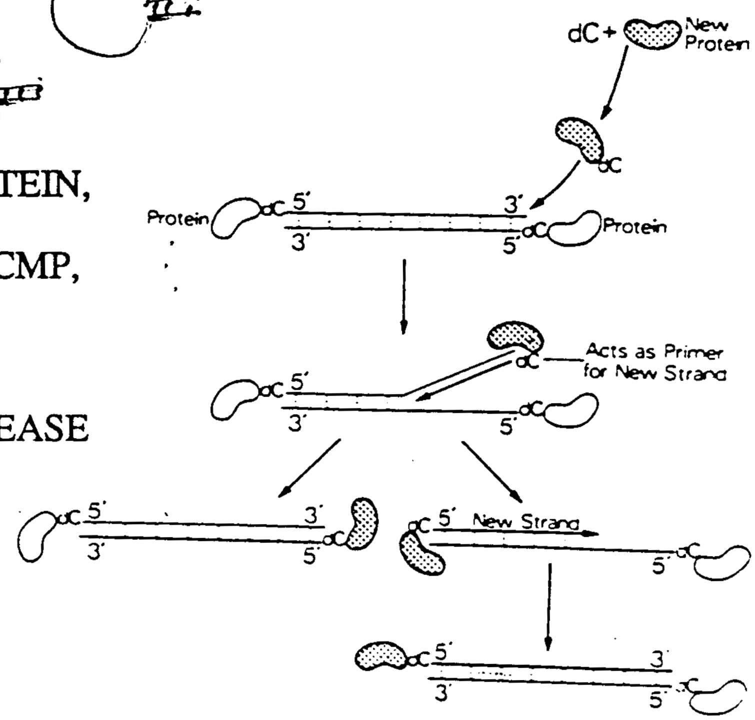
XYZ (e.g. HEXON)

AAA

PRINCIPLES OF ADENOVIRUS DNA REPLICATION

- VIRAL DNA POLYMERASE (PRODUCT OF E2 GENE)
- ORIGIN AT ENDS
- ENDS ARE INVERTED REPEATS





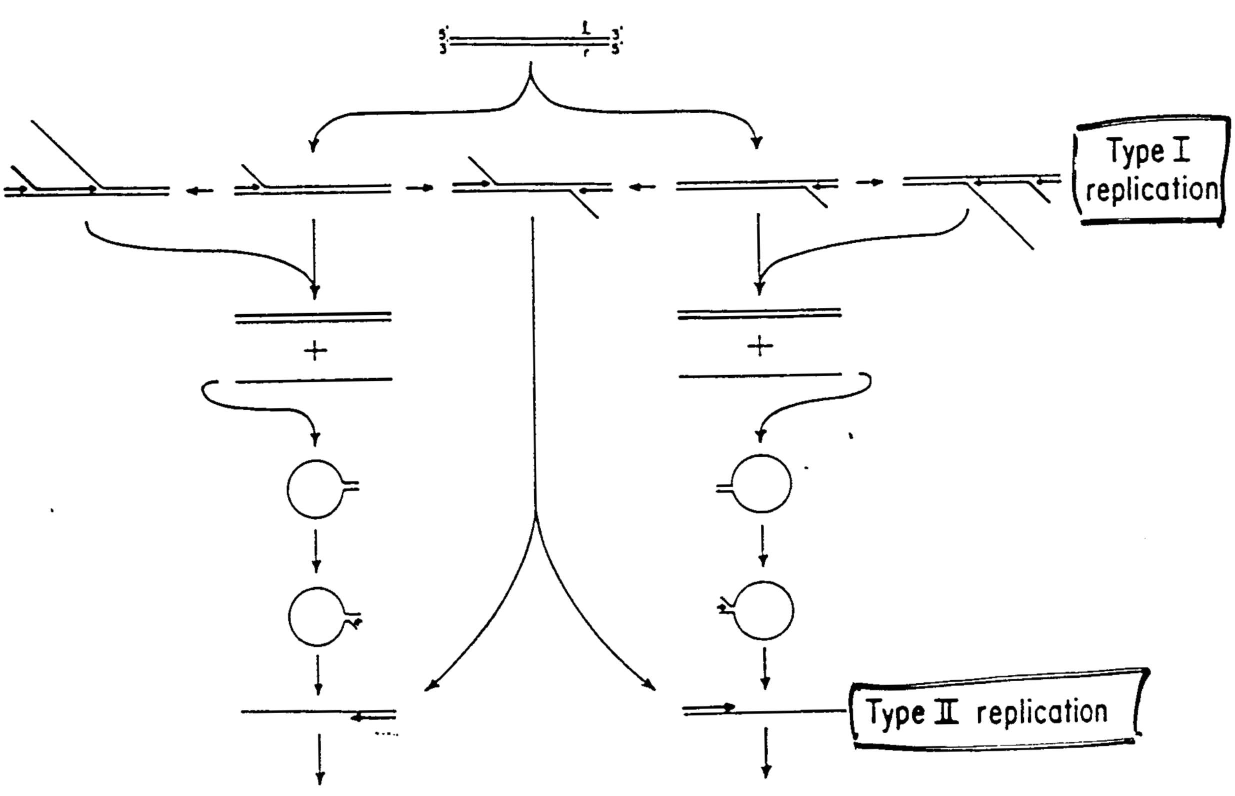
PRIMER IS VIRUS-CODED PROTEIN,

LINKED TO 5'ENDS BY SER-dCMP,

ENCODED BY E2

PROCESSED BY VIRAL PROTEASE

DISPLACEMENT (TYPE I) AND REPLACEMENT (II) SYNTHESIS



ADENOVIRUS DNA REPLICATION IN VITRO

- FIRST SUCCESSFUL IN VITRO SYSTEM:

  ADENO DNA WITH TP

  INFECTED CELL EXTRACT, dNTPS, ATP

  USE BUDR TO SHOW SYNTHESIS

  OBSERVE INTERMEDIATES BY EM

- INITIATION IN VITRO

- DEFINITION OF ORI FROM CUT PLASMID

- PURIFICATION OF FACTORS FROM INFECTED CELL EXTRACT:
  - P140: VIRAL DNA POL
  - P80: PRE-TERMINAL PROTEIN (PRIMER)
  - NF1 AND NF III FROM UNINFECTED NUCLEAR EXTRACT

    FOR INITIATION
  - P72 (VIRAL SS DNA BINDING PROTEIN) AND NF II (TOPO?)

    FOR ELONGATION

OTHER ILLUMINATING OR PROVOCATIVE FEATURES

$\boxed{\text{EIB}}$--->55KD AND 19 KD PROTEINS

55KD PROMOTES EGRESS OF VIRAL RNA FROM NUCLEUS

AND BLOCKS EGRESS OF HOST RNA

(ALSO BINDS P53....PER LATER LECTURES)

19KD LIMITS DNA DEGRADATION AND CYTOTOXICITY

$\boxed{\text{E3 PROTEINS}}$ MODULATE HOST RESPONSES

(E.G. BIND EGF RECEPTOR, INHIBIT LYSIS BY TNF,

BINDS MHC CLASS I IN ER)

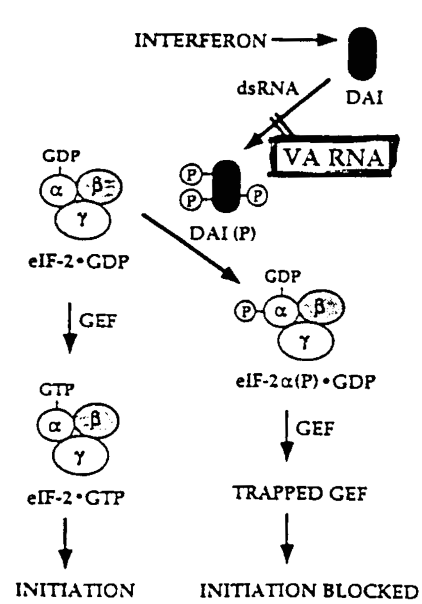
$\boxed{\text{E4 PROTEINS}}$ PROMOTE ACTION OF E2F ON E2 REGION

$\boxed{\text{SMALL VA RNAS}}$ PROMOTE LATE TRANSLATION BY

PREVENTING ACTION OF IFN-INDUCED DAI KINASE

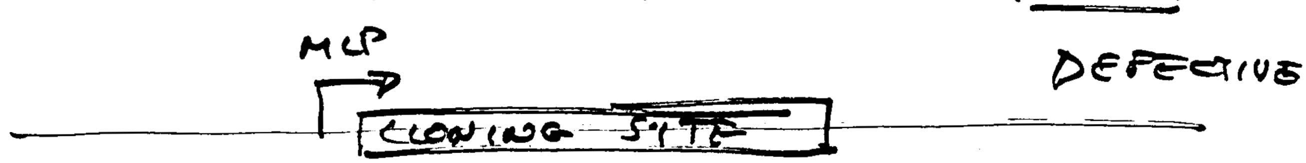
ON INITIATION FACTOR (eIF2)

VA I, VA II
POL III PROD.
~140 NT
SECONDARY
STRUCT.



ADENOVIRUS VECTORS

- EFFICIENT ENTRY, REPLICATION, AND EXPRESSION (VIA MLP)



OR DISPENSIBLE EARLY REGION (E3) → NON DEFECTIVE

- DELIVERY DOES NOT REQUIRE REPLICATING CELLS,

  WIDE HOST RANGE (RECEPTOR) FOR ADENOVIRUS

- DEFECTIVE OR INACTIVATED ADENOVIRUS ALSO USED FOR

  GENE DELIVERY VIA ADENORECEPTOR AND ENDOSOMES

  (DNA-POLYLYSINE-ADENOVIRUS COMPLEX)

REPRISE: WHICH OF THESE VIRUSES IS MOST USEFUL FOR UNDERSTANDING CELLULAR DNA SYNTHESIS?

PARVOS: USE HOST POLYMERASE, BUT DNA SELF-PRIMES AND ENDS NOT RELEVANT TO CELL TELOMERES

ADENOS: USE VIRAL POLYMERASE, PROTEIN PRIMER, ATYPICAL ORIGIN, CONTINUOUS SYNTHESIS

POLYOMAS: T AG/ORI RELATIONSHIP MAY HAVE NO EQUAL, AND REPLICATION IS UNCONTROLLED.....

BUT REPLICATION FORK (LEADING AND LAGGING STRANDS), USE OF HOST ENZYMES AND FACTORS, INTERSECTION WITH CELL CYCLE COMPONENTS IMPLY STRONG SIMILARITIES WITH HOST REPLIC.

PAPILLOMAS: CONSIDER THIS QUESTION IN DISCUSSION....